

## On the Composition of the Blood and Plasma of Test Cows Fed on a Purified Diet with Urea and Ammonium Salts as the Sole Sources of Nitrogen

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Feeding experiments with milking cows on a protein-free feed containing urea and ammonium salts as the sole sources of nitrogen<sup>1-6</sup> have been continued successfully. As a result of increasing the nitrogen content of the purified feed (from 17–20 g N to 24–27 g N/kg organic substance in the feed), the milk yield has risen considerably. Test cow No. 6 (Metta) has already reached a production of 4217 kg milk per year (calculated as standard milk equivalent to 684 kcal/kg). The milk yield of the test cows during the years 1962–63 when lower amounts of urea were fed, varied from 1936 to 2745 kg of standard milk.

In general the annual milk yield of the test cows on the test feed seems to be 3500–4000 kg milk, calculated as standard milk. The fat and protein contents of the test milk (O-milk) have been considerably higher than those of milk produced on normal feed. Special attention has been paid in this laboratory to the protein composition of the O-milk. The amino acid composition of the total protein and casein of the O-milk is very similar to that of milk produced on normal feed. The fractionation of casein and whey proteins of O- and normal milk using different methods, for example, gel filtration and electrophoresis, showed similar fractions to be present in both (Syväoja and Virtanen<sup>7</sup>). Small individual differences may arise from genetic variations.

In the present communication, a preliminary report of a study of the compositions of the whole blood and plasma of the test cows and other normally fed cows is presented.

In chromatograms (an automatic amino acid analyser was used) of the free amino acids of the whole blood or plasma from the test and normally fed cows, 18 ninhydrin-positive compounds were consistently detected besides the common 24 compounds. Glutathione was detected and estimated quantitatively only in the whole blood chro-

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matograms. Except for cysteic acid and methionine sulfoxide, which could result from the oxidation of the respective amino acids, the other ninhydrin-positive compounds may represent some of the various intermediates in the metabolic pathways of the common amino acids.

In chromatograms of the total blood protein hydrolysates, other ninhydrin-positive substances were found in small amounts besides the common amino acids of the blood proteins. Some of them were not identified and may possibly partly have arisen from the common amino acids during hydrolysis.

Blood ammonia as well as plasma urea and total nitrogen in samples taken from the test cows 6 hours after feeding were at the same levels as in samples from cows on normal feed. There were small variations in samples taken at different times and from different cows, but no systematic differences could be attributed to the feed. No ammonia was found in the blood of the test cows when the determination was made immediately after sampling.

The amino acid composition of the whole blood protein was quite similar in both the test and control cows, except for minor variations in the individual amino acid residues from different cows. Also electrophoretic studies of the serum proteins of the test and control cows carried out in this laboratory (Syväoja<sup>8</sup>) revealed only very slight differences in the various protein fractions from individual cows or from the test and control cows.

Regarding the free amino acid composition of the whole blood and plasma, considerable differences were found between the test cows and the cows on normal feed. The levels of most of the essential amino acids were lower in the test cows than in the normally fed cows. During lactation, the levels of some essential amino acids, particularly histidine, were much lower than normal in the plasma of the test cows. Since milk proteins are synthesized in the mammary gland mainly from the free amino acids of the blood, our results show that the mammary gland has a surprising capacity to take up amino acids necessary for the biosynthesis of the milk proteins from the circulating blood even when the amino acids are present at much lower concentrations than usual. However, the low concentrations of some essential amino acids apparently form bottle-necks in protein synthesis.

The total nitrogen content was generally lower in the whole blood of the lactating test cows than in the whole blood of the control cows. This was due to the fact that the hemoglobin content of the blood of the lactating test cows was lower than that of the blood of the control cows. On the other hand, the hemoglobin concentration of dry cows and heifers on the test feed was relatively high and at a normal level. Whether the low concentration of histidine or of other essential amino acids, a vitamin B<sub>12</sub> deficiency, or any other factors could be responsible for the impaired hemoglobin synthesis in the lactating test cows has not yet been elucidated. However, there is evidence that when the amount of nitrogen in the feed is increased, the contents of both hemoglobin and free essential amino acids in the blood increase.

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